ChIP protocol;
step-by-step guidelines and troubleshooting

Version 1.3 – CIGENE/NMBU

Important take-home messages

a. This documentation will be improved by the feedback of the users over the established protocols for ChIP experiment within the AQUA-FAANG project.
b. To each user, we recommend first you to through the entire protocol of ChIP (Day 1, 2 and 3) with liver samples.
   We warn you TO NOT PROCESS ALL YOUR SAMPLES with Day 1 Protocol even after having experienced good recovery of chromatin and good sonication profile. Indeed, a good sonication profile does not always guarantee the quality of the epitopes of the proteins bound to the DNA: Therefore, it is MANDATORY TO PROCEED UNTIL THE END OF DAY 3 with at least one sample (i.e. liver) to confirm the efficiency of the immunoprecipitation (i.e. the percentage of recovery).

Day 1

Tissue disruption

a. The recommended amount of tissue has been determined in salmon. It can be adapted to the species and available quantity of tissue. If the initial material is under 50 mg, we recommend reducing the buffer volume to 1 ml to avoid loss and excessive dilution of material, thus preventing a correct assessment of nuclei quality and quantity in step 4.
b. The indicative weight given in the protocols should allow you to recover enough quantity of chromatin to perform all the 5 different immunoprecipitations once, with a common input.
c. When a mortar is used, a pre-cooling of the mortar on dry ice helps the tissue disruption. This is especially mandatory for tissues that we can qualify as hard due to their structure. Indeed, a good disruption of the tissue is necessary to have a better recovery of nuclei.
d. The use of 2-ml or 15-ml dounce homogenizers didn't show any effect on the protocol itself. However, in general, the number of strokes by pestle A and/or B can be person dependent. Indeed, the strength and the habit to use these kinds of device makes a difference. The main common guideline is to stop as soon as no more resistance is felt.
e. The counting step (step 4) is essential for the sonication step. Please don't avoid it.
f. If you use Trypan blue, be aware that the colorant can precipitate. To avoid any problem of visualization and quality check of your nuclei, we recommend you to not shake the Trypan blue bottle before use. Also, you can filter a part of the colorant before use (filter a higher volume of colorant than what you need).
g. At step 5; be sure how to use accurately your cell counting chamber. Don't forget your dilution factor and change the calculation if your working solution is less than 5 ml at that step for specific reasons.
   For the Thoma cell (16 squares = 1 mm^3 = 1 µl);
   nb of nuclei inside the 16 squares* x 10 (dilution factor) x 1000 (conversion in ml) x 5 ml (working volume) = total nb of nuclei
   *If nuclei concentration is too high, count 3 squares instead of 16, calculate the mean nb, and multiply it by 16

Crosslinking

a. LoBind tubes are recommended to avoid any loss of material due to binding to the tubes, at all steps.
b. Crosslinking can be done with a preparation of formaldehyde from powder or from a dilution of a concentrated liquid solution (37%). To some extent, no difference in our laboratory has been observed between the use of these two types of solutions. Depending of your habit in the lab, you can also add the concentrated solution

of 37% directly to your samples in solution in PBS, by respecting a final concentration of 1% (135 µl for a volume of 5 ml).

c. The formaldehyde solution must be brought to room temperature for optimal efficiency.
d. Our lab did not compare the effect of quenching with glycine and with Tris. Nevertheless, the quenching with glycine has been chosen because it is still the most frequently used.
e. No experiment has been conducted in our laboratory to know the optimal ratio of nuclei with the volume of formaldehyde solution. However, it is common to find in the literature 10 million of cultured cells by ml of solution of formaldehyde 1 or 2%. Therefore, and based on the mean number of nuclei recovered by tissue, we recommend you to split in two tubes if the total number is over 24M of nuclei.
f. For smaller pieces of tissues, the centrifugation can be done with higher speed and longer time if loss of material is observed in the supernatant. Furthermore, skipping the second cleaning (steps 15 to 17) and staying in a working solution of 1ml can help to reduce loss of material if the starting material is critical.
g. After the decrosslinking and quenching step, keep your sample cold at any time, especially during the sonication process (see above in the section "Sonication").

Sonication

a. The sonication buffer helps the sonication process. The volume of sonication buffer can be adapted to the type of sonicator used and the number of nuclei you recover. This step is critical to recover the best profile of sonicated chromatin (200-700 bp size, with a mean size of 350-400 bp and a recovery of 70% minimum of the chromatin in that area).
b. The sonication parameters (number of cycles, cycles parameters) must be adapted to the material you use.
   To assess what are the best parameters for your own sample and material, we recommend following this step-by-step systematic procedure;
   1. Prepare your material according to the recommendations of your provider and follow the basic instructions for how to use your material and find the best parameters.
   2. Test for example a dilution of 2 million of nuclei by 200 µl of sonication buffer or 4 million in 400 µl
   3. Test sonication parameters which allow a balanced proportion of sonication time and rest time (for example, 1 sec ON / 1 sec OFF, 10 sec ON / 10 sec OFF… until 30 sec ON / 30 sec OFF). If possible, execute cycle no longer than 1 minute in total (periods ON and OFF included). Be aware that for some devices, 30 sec ON means 30 sec ON and 30 sec OFF = a total of 1 min by cycle, while, for others, 30 sec ON means a continuous 30 sec sonication, and then a resting time.
   4. Test a number of cycles between 12 and 20. For time management, 20 cycles become too much time consuming, and you can address other parameters at the sonication step (for example, intensity). Furthermore, the longer/the stronger you sonicate, the harder the immunoprecipitation can be (see next comment).
   5. You can test different number of cycles from the same sample, by recovering 20ul aliquot. But you also must replace the removed material by sonication buffer to respect the same total volume. Be also aware that, in that procedure and if working with 400ul, you remove 5% of your material.
   6. If the sonicator probe width is 2mm, test intensity between 40 and 55%. Over 55%, you might still recover a good sonicated chromatin. However, the epitopes of the proteins you will target at the immunoprecipitation step can be destroyed. Recommended volume of sonication varies with probe width.
c. In step 29, recover the maximum of the volume while avoiding any contamination from the pellet containing cellular and nuclear debris, which can alter the immunoprecipitation process.
d. Your tubes should be maintained in a cold environment all along the process; stay aware about the temperature of your cold bath if you work with sonicators in cold bath, and about the quality of the water you use for it, or use a metallic eppendorf holder, immersed in ice, to avoid any alteration of the protein epitopes and chromatin in general.

Tissue-specific challenges

Muscle

We are still working on the development of the protocol. Two versions of protocols are in test

The sodium pyrophosphate promotes the dissociation of the actin and myosin filaments (Hamm, 1970) which will reduce the anchoring of the nuclei

Gonads (male/female, immature/mature)

We are still working on the development of the protocol. One possible protocol is in test

Head Kidney

Clumping of the pellet is recurrent. We recommended you to break the pellet with your tip while you are resuspending it. Also, we recommended you to transfer these clumps into your solution of sonication, while you perform the sonication

Definitions

- Paraformaldehyde (PFA) is the name of the crystals
- Formaldehyde is in solution (in PBS). Some PFA can be formed in that solution, that is why the solution has to be renewed
- Formalin is formaldehyde in solution with methanol, at 36.5-37%. The methanol is used to reduce the formation of paraformaldehyde

Version history

Version 1.3 – 09.10.2020	Text correction
Version 1.2 – 01.10.2020	Add of a version history section Add of the comments b and d in section “Tissue disruption” Add of the comments b in section “Crosslinking” Add of the section “Tissue-specific challenges” and comments Add of the section “Definitions” and comments Add of a chapter “Important take-home messages and comments